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Results: Interlaboratory Comparison Bioconcentration Tests Using Eastern Oysters

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Prepared for

Office of Pesticides
and Toxic Substances

Prepared by

Environmental Research
Laboratory
Gulf Breeze FL 32561

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RESULTS: INTERLABORATORY COMPARISON-BIOCONCENTRATION TESTS USING THE EASTERN OYSTER.

by

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ENVIRONMENTAL RESEARCH LABORATORY

OFFICE OF RESEARCH AND DEVELOPMENT

U.S. ENVIRONMENTAL PROTECTION AGENCY

GULF BREEZE, FLORIDA 32561

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ABSTRACT

This report summarizes the results of an interlaboratory comparison for bioconcentration (BCF) testing using the eastern oyster (*Crassostrea virginica*) and the organic chemicals pentachlorophenol (PCP), 1,2,4-trichlorobenzene (TCB), and p,p'-DDE. The mean BCFs and high to low BCF ratios (H/L ratio) for PCP, TCB and p,p'-DDE were 64 (2.4), 168 (2.3) and 52,600 (3.4), respectively. The test method (ASTM Draft 9) used for all three chemicals and four participating laboratories resulted in data with a mean high to low ratio of 2.7, which represents the extreme estimate of variability. The mean H/L ratios for the time to 90% depuration for all three chemicals was 3.3. Considering the varying degrees of experience between participants in conducting BCF tests, the wide geographic distribution between participants, and the different dilution water characteristics, the results were very reproducible.

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INTRODUCTION

Under Section 4 of the Toxic Substances Control Act (TSCA), the Administrator of the Environmental Protection Agency (EPA) can require environmental effects testing of a chemical substances if (1) the manufacture, use, distribution, or disposal of that substance may present an unreasonable risk of injury to the environment; or (2) the substance will be produced in substantial quantities, is expected to enter the environment, and there are insufficient data to predict the effects of the chemical substance on the environment.

When issuing a test rule for the performance of environmental effects testing, the Administrator must also provide test standards to be used in the development of test data. Before test standards are proposed, steps should be taken by the Agency to insure that data developed by each test standard are adequate and reliable.

The performance of interlaboratory, or "round-robin," tests is one way to validate individual test standards and determine the degree of variability among data developed by different laboratories that use the same methodology.

Because the Office of Toxic Substances' (OTS) Oyster Bioconcentration Test Standard was not completed at the time of the contract award, contractors were instructed to follow the American Society for Testing and Materials (ASTM) "Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs" (Draft 9). This method is similar to that in the proposed draft of the OTS Test Standard.

This report is intended to summarize and review the final reports of the laboratories that participated in the "round-robin tests." In evaluating the bioconcentration data, we examined laboratory notebooks, progress reports, and final reports to determine how closely each laboratory adhered to the specific procedures outlined in the ASTM test method.

Three contract laboratories and one EPA laboratory participated in the "round-robin" tests. Each contract laboratory, in order to participate, had to satisfy a number of technical criteria stipulated in the scope of work. Two major criteria were: (1) competence in gas chromatographic analysis of the selected chemicals, and (2) availability of suitable natural seawater for conducting the test under flow-through conditions.

Test chemicals used in the bioconcentration "round-robin" tests were pentachlorophenol (PCP); 1,2,4-trichlorobenzene (TCB); and 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE). Selection of these chemicals depended upon a number of factors: (1) each possessed substantially different bioconcentration potentials as predicted by their octanol/water partition coefficients; (2) ease of chemical analysis; and (3) low mammalian toxicity.

The test species selected for the bioconcentration study was the eastern oyster, Crassostrea virginica. This species is important economically as a human food source and ecologically in its wide distribution on the Atlantic

and Gulf Coasts; and it is valuable in monitoring programs as a pollution indicator due to its ability to bioconcentrate many organic chemicals.

A major source of variability in the interpretation of bioconcentration tests results from the chemical analysis of tissues and water for the respective chemicals. For this reason, we conducted an analytical chemistry performance evaluation for all participating laboratories (after awarding contracts) to identify any significant variability associated with chemical analyses in these studies.

Contaminated tissue and water samples were provided to each of the participating laboratories (Appendix A). Samples were delivered by messenger to each participant on the same day. Final reports were required no later than 30 days following the delivery of the samples. Only one participant (Lab 4) failed to satisfy this deadline.

The lead laboratory, the Environmental Research Laboratory (ERL), Gulf Breeze, Florida, (Lab 1) conducted a large number of analyses for each chemical (in water and tissue) to establish relative mean concentrations, relative standard deviations, and corresponding 99% confidence intervals (Appendix B). Results from participating laboratories are tabulated as mean concentration and standard deviation. Those values that grossly exceed the 99% confidence interval are marked with an asterisk. It is clear from these data the one participant, Lab 2, had difficulty with the chemical analysis. A survey of the reports submitted with the performance evaluation results (Appendix C) revealed good homogeneity of chemical methodology, except that Lab 2 selected not to use a derivatization method for pentachlorophenol analysis and subsequently encountered high background interference. The reasons for poor performance by Lab 2 in the analytical chemistry performance evaluation were not obvious from its report.

The participants received the results of the performance evaluation, and those deemed acceptable were allowed to proceed with testing. Participants displaying poor performance were cautioned about their respective methods and improvements were suggested. Lab 2 was required to use a derivatization method for pentachlorophenol. The final results of chemical analysis for the bioconcentration studies are contained in the final reports (Appendix D).

In addition to the performance evaluation package, each participant was provided information on the test chemicals to choose properly the test concentrations and sampling periods, as prescribed in the ASTM methods document. According to the ASTM method, concentrations of the test chemical in water should not elicit adverse effects on the test organisms and, therefore, should not be greater than 0.1 times the 96-hour (h) EC50 (based on reduced shell deposition). Consequently, contractors were provided with the oyster EC50 (based on reduced shell deposition). Consequently, contractors were provided with the oyster EC50 values for each chemical (PCP, 104 µg/l; TCB, >500 µg/l; and DDE, 14 µg/l). The lowest value allowed for the chemical in water (as required by the ASTM method) is three times the analytical detection limit for each chemical tested by the participating laboratory.

The sampling intervals for each chemical are dictated by the logarithm of the octanol/water partition coefficient (log P); the higher the log P, the longer the time between samples and, therefore, the longer the exposure. We provided each contractor with the estimated log P for each chemical (2.89 for PCP, 3.32 for TCB, and 5.69 for p,p'-DDE), as generated by Veith et al. (1979).

In addition, each contract laboratory was requested to provide the lead laboratory with control and experimental oysters (collected at steady state) from each test to determine lipid content. This procedure was suggested in the ASTM standard practice for compounds that are lipophilic.

The ASTM document specifies certain required test methods or conditions in order for a test to be considered satisfactory. These conditions are always associated with the word "must." For example, Section 10.9.3 states (in part) that "a group of organisms must not be used for a test if the individuals appear to be diseased or otherwise stressed or if more than 3% die during the 48 h immediately before the beginning of the test." Suggestions for good test practices are generally phrased in words such as "should," rather than "must." For example, Section 10.3.2 states (in part) that "in any single test, all molluscs should be from the same year class and the valve height of the largest mollusc should be no more than 1.5 that of the smallest mollusc." Each laboratory's final report was carefully scrutinized to determine if the "musts" in the ASTM document were fulfilled. If not, an attempt was made to determine if the test results were affected.

BIOCONCENTRATION TEST RESULTS

Pentachlorophenol, Trichlorobenzene and DDE Bioconcentration Factors

The calculated steady-state bioconcentration factors (BCFs) for the three chemicals tested by the four participating laboratories are listed in Table 1. Steady-state BCFs were provided for each chemical by all laboratories.

Pentachlorophenol (PCP)—Results of the PCP bioconcentration studies indicated that the steady-state BCFs varied from 34 to 1,294. The obvious disparity of Lab 4's BCF (1,294) with the other three prompted a request that Lab 4 verify its calculations. Lab 4 could not determine any errors. However, a review of the PCP data in their final report (Table A-9 of Appendix D-3) indicated that the measured PCP varied from 0.42 to 22.6 µg/l. This accounted for the extremely large standard deviation (3.75) about the mean measured PCP value of 2.15 µg/l (Table 2). Therefore, we deleted this test from further discussion in this report.

Labs 1, 2, and 3 provided PCP bioconcentration factors of 82, 34, and 76, giving a mean of 64 $\pm$ 26. The ratio of the highest BCF value divided by the lowest (the high to low ratio, H/L ratio) for the three labs was 2.4.

1,2,4-Trichlorobenzene (TCB)—The results of the TCB bioconcentration tests, (Table 1) indicate that the mean BCF for the four laboratores was 168 $\pm$ 65; the H/L ratio was 2.3.

Table 1. Calculated steady-state bioconcentration factors (BCF) for Pentachlorophenol (PCP), 1,2,4-Trichlorobenzene (TCB), and p,p'-DDE, using the eastern oyster, Crassostrea virginica.

LABORATORY	PCP	TCB	p,p'-DDE
1	82	147	42,652
2	34	115	48,602
3	76	264	91,826
4	1,294*	148	27,191
	$\bar{x}$ = 64	168	52,568
	Std. Dev. = 26	65	27,684
	H/L Ratio = 2.4	2.3	3.4

*Data not considered in $\bar{x}$, std. dev. and H/L ratio.

Table 2. Nominal and mean measured water concentrations (µg/l) of pentachlorophenol (PCP), 1,2,4-Trichlorobenzene (TCB) and p,p'-DDE for each laboratory in bioconcentration interlaboratory comparison studies.

LABORATORY	PCP		TCB		p,p'-DDE	
	Nominal	Measured	Nominal	Measured	Nominal	Measured
1	5.0	3.7 ± 1.4	5.0	4.4 ± 0.54	1.0	0.38 ± 0.19
2	10.0	9.5 ± 1.2	25.0	8.8 ± 1.9	1.0	0.26 ± 0.1
3	10.0	11.3 ± 1.7	50.0	52.1 ± 3.1	1.2	0.66 ± 0.31
4	N.R.*	2.15 ± 3.75	N.R.	4.2 ± 0.3	N.R.	0.43 ± 0.07

*Not recorded in final report.

DDE--The results of the p,p'-DDE bioconcentration tests (Table 1) show that the steady-state BCFs ranged from 27,200 to 91,800, indicating that the mean BCF for this insecticide was 52,600 ± 27,700. The H/L ratio was 3.4.

Table 2 compares the measured water concentration to nominal (intended) concentration for each laboratory and each test chemical. Nominal concentrations were not provided by Lab 4 in the final report.

Depuration rates (the rate at which test chemical were purged from oyster tissues when exposed to seawater free of the test chemical) were calculated for all participating laboratories and test chemicals except for Lab 4 and p,p'-DDE (Table 3). The time required for 90% depuration of PCP from the respective steady-state value ranged from 8 h (Lab 1) to 25 h (Lab 4), giving an H/L ratio of 3.1. For TCB, time to 90% depuration ranged from 17 h (Lab 2) to 90 h (Lab 4) giving an H/L of 5.3. For p,p'-DDE, depuration rates were calculated for only three laboratories (1,2 and 3), giving an H/L ratio of 1.6. Lab 4 depuration data indicated that there was no significant loss of p,p'-DDE over the 28-day depuration period. Reasons for this phenomenon are not clear, but Lab 4 was consistently higher in the time required to depurate all three test chemicals.

Analysis of the Adherence of Laboratories to the ASTM Methods and Scope of Work

Final reports from each laboratory involved in the bioconcentrations "round-robin" tests were carefully reviewed to determine if the "must" requirements of the ASTM document and the contract's Scope of Work were implemented. Tables 4, 5, and 6 address these requirements and their implementation.

Each laboratory, except Lab 4, provided sufficient information to make an adequate appraisal of diluent water quality and test water conditions used in the biconoentration tests (Table 4). The only information provided by Lab 4 was the temperature of the test water. For diluent water quality, Labs 1, 2, and 3 each measured salinity, pH, and dissolved oxygen (D.O.) and those values were within prescribed limits. Particulates were measured by Labs 1 and 3, but not by 2 and 4; only Lab 3 measured total organic carbon (TOC). Total organochlorines were measured by Lab 1 only.

Measurement of test-water conditions varied among laboratories (Table 2). Salinity was within prescribed limits (>12 g/kg) for Labs 1 and 2, but Lab 3 maintained salinity above 12 g/kg only during the PCP test. As stated earlier, Lab 4 reported only test temperatures in their final report. The report did state, however, that "open ocean seawater" was used for their test; from this, one could assume that salinity was >12 g/kg. The remaining test water conditions (namely, temperature, pH, and D.O.) were measured by Lab 1, 2, and 3 and were within the prescribed limits.

Assuming that total organochlorine pesticides in the test water (other than the test compounds) were sufficiently low that the oysters were not adversely affected, we would expect that only the low salinity data from Lab 3 might adversely affect the test results. Salinity in two of their tests

Table 3. Calculated time, in hours, to 90% depuration (from steady state) for pentachlorophenol (PCP), 1,2,4-Trichlorobenzene (TCB) and p,p'-DDE, using the eastern oyster, Crassostrea virginica.

LABORATORY	PCP	TCB	p,p'-DDE
1	8	24	582
2	9	17	654
3	17	30	960
4	25	90	NSD*
**H/L Ratio	3.1	5.3	1.6

*No significant depuration detected over 672 hours in DDE-free seawater.
**The greatest time to 90% depuration divided by the shortest time.

Table 4. Performance of each laboratory in adhering to diluent and test water condition requirements specified in ASTM bioconcentration test procedures. "Yes" designates compliance with the requirements; "No" designates that the required dilution water observations were not made, or that test water conditions were not within required limits.

	DILUENT WATER QUALITY*					
LABORATORY	Salinity	pH	D.O.	Particulates	TOC or COD	Total Organochlorine COD
1	Yes	Yes	Yes	Yes	No	Yes
2	Yes	Yes	Yes	No	No	No
3	Yes	Yes	Yes	Yes	Yes	No
4	NR**	NR	NR	NR	NR	NR

	TEST WATER CONDITIONS***			
	Salinity (>12 g/kg)	Temperature (< 28 C)	pH (<0.8 unit)	D.O. (>60% saturation)
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	No****	Yes	Yes	Yes
4	NR	Yes	NR	NR

* "Should" requirements in the ASTM procedure.

** NR = not reported in the final report

Table 5. Performance of each laboratory in adhering to ASTM bioconcentration test procedures regarding eastern oyster (Crassostrea virginica) size, loading, acclimation time, and mortality during acclimation and testing. "Yes" indicates compliance with the requirements; "No" indicates non-compliance or data were not reported.

LABORATORY	Oyster Size (largest <1.5 x smallest)	Mortality		Acclimation Time* (>4 days)	Loading* (>1 l/oyster/h)
		Acclimation (<3%)	Test (<10%)		
1	Yes	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes	Yes
3	Yes	Yes	NR**	Yes	Yes
4	Yes	Yes	Yes	Yes	No***

* A "must" requirement in the ASTM procedure.
** NR = not reported with final report.
***Loading was acceptable only for the pentachlorophenol test.

Table 6. Analyses of lipids (dry-weight) in oysters obtained from laboratories participating in the "round-robin" tests. Oysters were collected at steady state and analyzed individually.

LABORATORY	N*	Mean Percentage Lipids		
		Neutral	Polar	Total
1	5	2.4	-	-
2	8	3.7	4.1	7.8
3	8	6.0**	6.0	12.0**
4	8	3.6	5.2	8.8

* N = number of individual oysters measured to determine mean percentage lipid

** = significantly ($\alpha = 0.05$) higher in percentage lipid than labs 2 and 4, using ANOVA and Duncan's multiple range test.

dropped as low as 2 g/kg (Appendix D-2). Pumping rates of eastern oysters exposed to these low salinities were undoubtedly reduced; however, Lab 3 made no mention of reduced pumping rates or reduced deposition of feces or pseudofeces. The high steady-state BCFs associated with Lab3tend to indicate that the oysters were unaffected.

Lipid Analyses of Steady-State

Each contract laboratory was requested to provide ERL, Gulf Breeze with oysters collected at steady state from each test to determine the oyster lipid content. Oysters were received frozen and remained so until lipid analyses were conducted. Eight individual analyses were made on oysters from laboratories 2, 3, and 4 to determine polar, neutral, and total percentage-lipid (dry weight). Results of these analyses are listed in Table 6. Lab 1 (ERL, Gulf Breeze) lipid analyses were incomplete because procedures used to determine lipid differed from those used for the other laboratories (see Appendix F). (Also, Lab 1's oysters were used to identify potential toxicant-related effects on lipid content of which none was statistically different; therefore, were not used for comparison with other labs).

A comparison of the percent lipid values of labs 2, 3, and 4 (Table 1) with their respective steady-state BCF (Table 1) demonstrates a potential relationship. Lab 3's oysters contained significantly higher neutral and total lipids than those of labs 2 and 4; correspondingly, oysters of Lab 3 were also higher than those of the other two laboratories in the steady-state BCFs for all three chemicals tested. We did not attempt to adjust the steady-state BCFs for lipid content, because accurate wet weight could not be determined for the oysters. However, recommendations for changes in the ASTM procedure to utilize fully lipid data for BCF normalization follow in the Discussion section of this report.

DISCUSSION

The results of the bioconcentration interlaboratory tests indicate that the ASTM method can generate reproducible results. The mean H/L ratio for all three chemicals and four laboratories (excluding Lab 4 for PCP) was 2.7. Because replicate tests were not conducted within each laboratory, no estimate of variance could be determined. The mean H/L ratio of 2.7, however, indicates good reproducibility, considering the varying degrees of experience among laboratories in conducting bioconcentration tests, wide geographic distribution among laboratories, and different dilution water characteristics. In addition, lipid content of oysters can vary by a factor of ten. For lipophilic chemicals this can cause bioconcentration factors to vary proportionately. Lipid content of oysters in these tests, where available, varied little.

Depuration rates for each chemical generally varied more than the BCF. The H/L ratios of the time (in hours) required for 90% depuration of PCP, TCB, and p,p'-DDE were 3.1, 5.3, and 1.6, respectively. Laboratory 4 was consistently high in the depuration rates of all three chemicals.

Several problems in the ASTM bioconcentration test procedure were evident to us and to some of the contract labs. One difficulty was the precise determination of steady state during the test. Currently, the procedure recommends the use of Analysis of Variance (ANOVA) to determine when steady state has been attained (i.e., to determine when there is not significant difference in residues from one sampling interval to the next). The difficulty encountered was the occurrence of a wide range of chemical residues in oysters during a single sampling period. This wide variability about the mean caused difficulty in reliably estimating steady-state. We have no firm recommendations for solving this problem during this critical portion of the test (critical in that one is unsure exactly when to start depuration), since the oyster's biological variability is the basic cause. The only recommendation we can support is to extend the exposure portion of the test one sampling period beyond the minimum period to ensure that steady state has been reached or to increase N, the number of samples taken at each sampling period.

Another difficulty encountered by contract laboratories was the lack of accessible satatistical models for analyzing bioconcentration data. ERL, Gulf Breeze analyzed all data for uniformity (NONLIN); but, in general, models such as BIOFAC and NONLIN are not universally available and often require a professional statistician for interpretation. We recommend that OTS, in concert with the research laboratories, provide specific statistical packages to be made available to contract laboratories.

The ASTM bioconcentration method could be improved by including specific techniques for lipid analyses. We found substantial variation in the water content of oysters submitted by the various contract laboratories due to sampling and storage differences and could not normalize BCF values, which are based on wet weight, by using lipid values, which are based on dry weight. We recommend use of standardized oyster sampling procedures (Appendix E). When oysters are sampled for lipid analysis at steady state, the wet weight should be determined at the time of sampling; oysters should be freeze-dried and reweighed before total lipids are determined (see Appendix F).

LITERATURE CITED

Veith, G.D., D.L. Defoe, and B.V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Bd. Canada. 36: 1040-1048.

APPENDIX A

ANALYTICAL CHEMISTRY PERFORMANCE EVALUATION

APPENDIX A

November 7, 1979

U.S. Environmental Protection Agency
Environmental Research Laboratory, Gulf Breeze
OTS Bioconcentration Round Robin
Analytical Chemistry Performance Evaluation

Samples Enclosed:

1. 1,2,4-Trichlorobenzene--test material-20 gm.
2. Pentachlorophenol--test material-20 gm.
3. p,p'-DDE--test material-2x1 gm.
4. 1,2,4-Trichlorobenzene--water spike (acetonitrile)-5 ml.
5. Pentachlorophenol--water spike (acetonitrile)-5 ml.
6. p,p'-DDE--water spike (acetonitrile)-5 ml.
7. Oyster Tissue Extract--blank (acetonitrile)-2x100 ml.
8. 1,2,4-Trichlorobenzene--tissue extract (acetonitrile)-100 ml.
9. Pentachlorophenol--tissue extract (acetonitrile)-100 ml.
10. p,p'-DDE--tissue extract (acetonitrile)-100 ml.

CAUTION: Refrigerate samples when not in use. Prior to use, allow bottle to reach room temperature. Agitate bottle prior to sampling.

Analytical Standard Preparation: Prepare all analytical standards for this study from the test material provided.

Water Sample Preparation: Add 1000 ml of test seawater to a 2000 ml separato funnel. Add exactly 500 microliters of appropriate water spike concentrate to the separatory funnel and mix well. The sample is ready for extraction an analysis. A blank 1000 ml seawater sample should be analyzed concurrently for background.

Tissue Sample Preparation: Add 75 ml of aqueous 2% sodium sulfate (weight/volume) to a 125 ml separatory funnel. Add exactly 10 milliliters of appropriate oyster tissue extract to the separatory funnel and mix well. The sample is ready for extraction, cleanup, and analysis. Blank oyster tissue extract samples should be analyzed concurrently for background.

Reporting Results: Report, in detail, the exact procedures used in this study for extraction, cleanup, and analysis. Do not correct for background or fortified recoveries. For water samples, report results in micrograms per liter to the number of significant figures that your method will allow.

For tissue samples, report results in micrograms per gram, whereby 10 milliliters of extract is equivalent to 5 grams of wet tissue, and to the number of significant figures that your method will allow. If replicate samples are analyzed, report each result. In the final report, include all original chromatograms (especially blanks), computer reports, personnel data sheets, and calculations.

Report in detail the exact procedures to be used for water and tissue analysis in the proposed bioconcentration studies with 1,2,4-trichlorobenzene; pentachlorophenol; and p,p'-DDE. Include results of spike samples and limits of detection. Further questions and final reports should be addressed to:

Mr. Steven Schimmel
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, Florida 32561
904-932-5311

Final reports for this performance evaluation must be received by us no later than December 7, 1979. Actual bioconcentration tests may begin after notification of successful completion of this performance evaluation by Mr. Schimmel.

APPENDIX B
CHEMICAL PERFORMANCE EVALUATION

APPENDIX B

OYSTER BIOCONCENTRATION ROUND ROBIN: RESULTS OF CHEMICAL PERFORMANCE EVALUATION

	Pentachlorophenol		Trichlorobenzene		p,p'-DDE	
SUBSTRATE	WATER	TISSUE	WATER	TISSUE	WATER	TISSUE
Intended Concentration	4.0 µg/ℓ	0.50 µg/g	5.0 µg/ℓ	1.0 µg/g	1.0 µg/ℓ	1.0 µg/g
Lab 1						
$\bar{x}$	3.8	0.35	4.7	0.67	0.91	0.86
Std. Dev.	0.92	0.062	0.048	0.034	0.030	0.046
N	21	14	13	13	13	15
99% C.I.	2.0-5.6	0.23-0.47	3.8-5.6	0.60-0.74	0.85-0.97	0.77-0.95
Lab 2						
$\bar{x}$	4.3	0.63*	57.*	0.65	1.7*	0.96
Std. Dev.	0.39	0.070	4.4	0.019	0.17	0.017
N	5	5	4	4	4	4
Lab 3						
$\bar{x}$	3.4	0.37	3.9	0.74	0.7	1.0
Std. Dev.	0.12	0.017	0.12	0.046	0.14	0.0058
N	3	3	3	3	3	3
Lab 4						
$\bar{x}$	3.0	0.31	4.2	0.51	0.85	0.66*
Std. Dev.	0.071	0	0	0.064	0.014	0.021
N	2	2	2	3	2	2

*Substantially different from values derived by Lab 1 (control lab).

APPENDIX E

Method for Sampling Oysters

The oyster should be opened with an oyster knife by separating the valves at the hinge. Once separated, the knife should then sever the adductor muscle at the point of attachment to the left (upper) valve.

Care should be taken not to penetrate the mantle. Discard the upper valve and shake the right valve (with attached living tissue) three times to remove excess water. Sever the adductor muscle at the attachment to the right valve. The living oyster tissue should then be intact, free of the valves, and ready for analysis.

APPENDIX F

Methods for Lipid Analyses

All tissues were lyophilized to dryness prior to extraction. Neutral Lipids - similar to the tissue method of Schimmel et al. (1979) for the analysis of EPN and leptophos, except that the combined hexane extract was not cleaned by florisil, but was dried to constant weight as percentage lipid by dry weight. Marine Sci. 22 193-203 (1979). Polar lipids, Total lipids - homogenized by the method of Schimmel et al. (1979) with a mixture of chloroform/methanol/water, as suggested by Bligh, E.G. and W.J. Dyer (1959) Can. J. Biochem. Physiol. 37, 911. For polar lipid determination, the tissue was first extracted for neutral lipids, then for polar lipids.

A general reference:

Laboratory techniques in biochemistry and molecular biology

General editors: T.S. Work and E. Work. Techniques of Lipidology: Isolation, analysis, and identification of lipids Morris Kates, North-Holland/America, Elsevier, 1972